THE UNIVERSITY OF ALBERTA

"PERIPHERAL NERVE IMPLANTATION STUDIES IN EXPERIMENTAL PARAPLEGIA"

A Dissertation
Submitted to the School of Graduate Studies
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for the Degree of Master of Science (Surgery)

- Department of Surgery -

by

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SYNOPSIS

In the introduction a historical review and appraisal of the literature on central nervous regeneration and reinnervation procedures in the spinal cord of mammalia is presented.

Chapter II describes the methodology. Reinnervation of the distal stump of the transected spinal cord is attempted by implanting in it a centrally connected sympathetic nerve trunk or single or multiple intercostal nerves. While intercostal nerve implantation has been previously studied, sympathetic nerve trunk implantation is a new procedure.

Results and observations are discussed in Chapter III. It was found that both sympathetic and intercostal nerve implants can appreciably improve the functional performance of dogs which had spinal cord transections. De novo observations on muscle tonicity due to sympathetic de-afferentation are presented. Reinnervation of the distal stump is histologically substantiated.

In Chapter IV the results are critically examined. The probable mechanism by which regenerating neurofibrils of the nerve implant influence reflex activity is discussed.

The final chapter is devoted to the conclusions of this study.

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CHAPTER I

- I N T R O D U C T I O N -

Investigations on central nervous system regeneration have one final objective; the facilitation of functional return following trauma. This premise is especially true for lesions of the spinal cord.

A little over a century ago, Waller described the morphological changes observed subsequent to lesions of peripheral nerve cells and axons. His logical and well constructed conclusions were however not uniformly accepted as considerable controversy existed concerning this phenomenon. Two diagonally opposed views were current, championed by their proponents, the monogenists and polygenists. Monogenists, principally Ranvier, Stroebe and Kolster subscribed to the neuron doctrine, recognizing the neuron as the trophic unit and the inability of the distal segment of a transected axon to regenerate as a separate unit. In contradistinction, Phyllipeaux, Vulpian and notably Bethe believed that in young animals autogenous regeneration of the peripheral segment of a severed axon accounts for restoration of axonal continuity. They nevertheless conceded that for adult animals "the regeneration of the peripheral stump involves the co-operation of the fibres of the central stump." (Cajal, 1928)

Golgi introduced the technique of silver salt precipitation for nerve stains in 1880, and by 1905 enough conclusive evidence was accumulated by Perroncito, Marinesco and Lugaro of the Italo-Spanish schools to dis-

credit the polygenist faction.

Waller's thesis and Waldeyer's neuron doctrine became universally accepted.

In 1914, climaxing eight years of intensive experimentation, Ramon y Cajal compiled a then obscure monograph on degeneration and regeneration of the nervous system, now recognized as one of the classics in neurology. Cajal stated that traumatized central neurons of mammalia are "able to emit ramified sprouts" which however fail to make effective connections. This inability of central nervous system neurons to regenerate without establishing functional connections was referred to as the concept of "abortive regeneration."

In the interim it was also established that central nervous regeneration does occur in phyla lower than mammalia (Lorente de No 1921, Pearcy and Koppanyi 1924, cf. Sugar and Gerard 1940), but as the phylogenetic scale progresses, a corresponding decline in nervous regenerative potential is apparent.

Gerard and Koppanyi (1926) transected spinal cords of rat embryos in utero, 1 - 8 days prior to birth, as well as the cords of recently born and young adult rats. Of the first two groups 3 of 36 showed motor and sensory improvement, while no improvement was observed in the older rats. This study had no histological substantiation.

Hooker and Nicholas (1927, 1930); Nicholas and Hooker (1928) used cautery to produce transection in over 300 rat fetuses. Although histologically "not the slightest evidence of regenerative phenomenon was observed" these rats showed reflex activity not unlike those observed in normal fetuses. These workers postulated that transmission of impulses past the spinal cord lesion occurs via collateral pathways facilitated by mechanical pulling of tissues, thus initiating reflexes in the hind limb.

Gerard and Grinker (1931) repeated some of the previous cord section studies on embryonal rats in utero, remarking that although there was restitution "of a great deal of function .....cannot conclude that a significant central regeneration is possible."

During the next decade numerous publications appeared on the behavior of spinal animals, but only two workers dealt directly with the problem of spinal cord regeneration. Migliavacca (1930) observed functional return in two foetal and newborn rats following complete rachiotomy, while Marburg (1936) observed no nervous regeneration in dogs which had one or both lateral columns sectioned.

Three principal objections may be raised against the mentioned earlier studies:

1) Fetal or very young rats were used. In the rat, neuroblasts continue postnatal differentiation for 20 - 22 days (Addison 1911, Allen 1912, Sugita 1918 cf. Windle 1956).

2) Improper visualization of transection ("Blind section").

If 2 - 5% of the ventral funiculus only remains intact, retention of varying degrees of voluntary movement is present (Windle, Smart, Joralemon 1955).

3) Inadequate or no histological confirmation of regeneration.

In an elegant study, Sugar and Gerard (1940) presented unequivocal evidence for central nervous regeneration in the transected spinal cords of 3 to 5 week old rats. Transection was adequately visualized, histological and stimulation methods conclusively designed. Functional return was noted in 13 animals after a latency period of ca. one month, stimulation of the cerebral peduncles producing hind leg movements. In some of the rats, predegenerated peripheral nerve segments and particles of muscle were used as implants across the site of transection of the spinal cord.

LeGros Clark (1940) confirmed the differentiating potentials of embryonic nervous tissue transplants in rabbit brain, thus concurring with Dunn (1917), but in two publications (1942, 1943) denied the regenerative ability of intrinsic adult neurons.

Experimenting with adult dogs and cats, Brown

and McCouch (1947) noted that "no trace of functional regeneration was observed" following transection of the spinal cord. They employed protective tissue wrappings (amnion, gall bladder, aorta) around the site of transection and attempted to stimulate nervous neoformation by painting the severed cord ends with an emulsion of predegenerated peripheral nerves. The final conclusion of the study implicated the dense collagenous scar at the site of the lesion as the main barrier to nervous regeneration.

Davidoff and Ransohoff (1948) attempted to prevent scar formation at the site of spinal cord transection by placing gelatin capsules and gall bladders over the stumps of the severed spinal cord in cats, but obtained no evidence "on any regenerative power from the upper or lower ends of the transected spinal cords." However, Freeman, Finneran and Schlegel (1949) in a series of 500 young adult rats observed functional recovery in a "fairly high" percent of the animals surviving beyond two months. These workers observed regenerating spinal root and internuncial components in successful preparations.

Absence of functional return in 159 adult rats was reported by Bernard and Carpenter (1950) following transection of the spinal cord. Although some regenerating fibers were observed, possibly of dorsal and ventral root origin, their

penetration of the scar tissue was so feeble that these workers concluded that "connective tissue was not of primary importance in retarding their growth."

Freeman (1952) reinvestigated the regenerative potentials of the transected spinal cord in ca. 2,000 rats, pups and kittens. Anatomical and physiological studies substantiated functional recovery, and implicated spinal neurons. Pia mater, arachnoid mater, pulpified cord tissue, nerve grafts and plastic covering were used in an effort to promote regeneration. However these methods only increased glial scarring.

The pyrogenic bacterial polysaccharide, Piromen, was used by Windle and Chambers (1950) to influence glial scar formation in the transected cords of adult dogs and cats. Although no functional recovery was observed (longest survivor 139 days), sections showed the dense glial scar to be absent. Regenerating intrinsic neurons and neurons from spinal radices penetrated the loose scar, invading the distal segment of the spinal cord. Gokay and Freeman (1952) confirmed in rats the efficacy of Piromen in reducing glial scarring, which reduction allows regenerating axons to make functional connections in the distal segment of the spinal cord.

Scott and Clemente (1951, 1955) performed electrophysiologic studies on 8 Piromen-treated cats 7 - 17 months following transection of the spinal cord at level T-12.

These workers were able to locate regenerating fibers by means of pick up electrodes and recorded evoked potentials 30 mm. below the level of the transection. The recorded potentials were from 11 - 42% of the height of responses similarly obtained in controls.

Conduction velocities, combined action potentials implicated the lateral funiculus as the site of regenerating fibers. This assumption was indeed verified by localizing regenerating fibers in histological preparations in this region. Liu and Scott (1958) advanced further electrophysiological proof for regeneration in the spinocerebellar tract of cats subjected to crush lesions at levels C-4 and T-12, subsequently treated with Piromen. Histological confirmation of regeneration was presented.

Lance (1954) however observed no regeneration of pyramidal axons of adult cats following transection and Piromen administration. Arteta (1956) while conceding the scar modifying actions of Piromen, was unable to see functional regeneration of the spinal cord in 5 adult cats with incomplete spinal cord transection in the lumbodorsal region. Regeneration, abortive in nature, was confined to posterior root neurons. Hess (1956) transected the spinal cords of fetal guinea pigs in utero and observed morphologic changes chronologically for 12 days post-natally. He states didactically, "In short, the nerve tissue of the mammalian

central nervous system appears to lack inherently the ability to regenerate under ordinary circumstances." Windle et al (1956) concluded that "some regeneration of intraspinal neurons occurred in spinal monkeys under therapy with Piromen which in some way played a permissive role." No functional recovery was observed.

Intrathecal trypsin administration in 48 dogs with hemisection of the spinal cord reduced glial scar formation and thereby facilitated functional improvement (Freeman 1958).

Bassett et al in a series of communications (1957a, 1957b, 1959a, 1959b) described an ingenious tubulation technique for cut peripheral nerves and transected spinal cords. Using a monomolecular porous plastic filter sheath of HA Millipore, they were able to demonstrate reduced glial scar formation and "orderly axonal bridging of the completely transected spinal cord." No functional recovery was observed in the adult spinal cats studied.

Turbes et al (1960) irradiated the site of spinal cord transection in adult dogs, using 1,000 to 2,000 r. They observed an effective diminution in the amount and density of the connective tissue scar. The regenerating neurofibrils traversed this scar easily, establishing connections in the distal segment of the spinal cord. "Co-ordinated activity of the hind quarters with the fore limbs" was ascribed to this central regeneration.

Most of the enumerated experiments agree in at least one significant point, viz. the regenerating neurofibrils in the transected spinal cord are predominantly of spinal root origin. (Sugar and Gerard 1940, Windle and Chambers 1950, Bernard and Carpenter 1950, Gokay and Freeman 1952, Arteta 1956). Dorsal root fibers demonstrate an especially high degree of regenerative potential (Turbes and Freeman 1961).

As early as 1943, Tower postulated the functional regenerative ability of anterior spinal roots. Freeman (1949) sectioned the spinal roots of dogs from L-5 caudally, anastomosing them in a purposely random fashion. In one dog almost complete function returned after a period of one year. Hypothesizing that "any spinal nerve root could be regarded as an internuncial neuron which through alterable design had become associated with a distal motor element rather than its proximal motor pool" Freeman (1958) postulated that it is only necessary to establish cortical connections with the reflexly functioning distal spinal cord to have return of voluntary function. This thesis also presupposed that little or no specificity exists in a nerve root. Two previous studies (Sperry 1947, Freeman 1952) appear to support this latter contention. In subsequent experiments Freeman and his associates observed almost complete functional return in the majority of adult dogs, which had a centrally connected intercostal nerve

inserted into the distal segment of the transected spinal cord (Turbes and Freeman 1958, Jakoby et al 1960). The animals in the last two experiments mentioned were concurrently treated with Piromen and x-ray radiation. Of a series of 30 dogs, 25 developed "reflex standing" and "reflex walking" 10 days to two weeks after insertion of a single intercostal nerve into the distal segment of the transected spinal cord. At the implantation site, histology showed an abundance of regenerating nerve fibrils localizing closely to intact central axons. Following section of the nerve implant 5 animals reverted to complete paraplegia. Excessive proliferation of connective tissue followed multiple implants, this procedure showing no advantage over single implants. Cortical stimulation of successful preparations produced a volley of impulses in the muscles of the hind limbs, which activity disappeared completely after severing the anastomosing nerve. The procedure of intercostal nerve implant into the transected spinal cord was used in an applicable human case by Freeman (1960). Although histological evidence of regeneration was present, the premature death of the patient from other causes terminated the study - prior to his demise no objective functional recovery was apparent.

Rossiter and Berry (1959) conducting in vitro experiments on nerve regeneration, postulated that among other substances, ethanolamine - 1,2 increases the potential of a previously degenerated peripheral nerve for remyelinization.

In consolidating the experimental evidence reported since 1940 a few controversial points have to be resolved.

1) Studies utilizing young animals generally report a higher percentage of regeneration than ones involving adult animals.

It is unlikely that the C.N.S. of younger animals possesses more plasticity in neoformation than adults. More feasible is the already mentioned fact that neuroblasts, immature at the time of trauma, subsequently develop at their normal rate of growth and thus account for regenerative phenomena. The work of Hess (1956) may find some discredit in its short duration as well as the method of transection used.

2) Not all studies using Piromen report successful central nervous regeneration.

Biological experimentation does not conform to the regularity of physical sciences. Improper operative techniques, inadequate care of the animals may defeat an otherwise excellent experimental design. One should realize as well the variable potency of drugs. Storage at temperatures higher than 2°C. may render pyrogens entirely ineffective.

3) Frequently histology of untreated animals shows regeneration of central neurons similar to that observed in Piromen treated animals.

In spinal animals urinary infections are common; encountered almost uniformly in rats. The microorganisms involved have pyrogen producing properties and thus produce Piromen-like effects as reported by Freeman (1955) and Windle et al (1956).

4) Is functional recovery a manifestation of reflex spinal activity or a result of axonal neoformation with subsequent establishment of synaptic connections?

Although Shurrager (1951) appeared to present evidence in favor of spinal conditioning, his thesis was justifiably questioned by Freeman (1952) and Littrell (1955) after discovery of unsectioned fibers in histological sections of the successfully conditioned animal. The work of Freeman (1958) Scott and Clemente (1951, 1955) would militate against accepting reflex activity as the sole factor responsible for functional recovery in spinal animals.

5) Do regenerating fibers truly represent neoformation or merely residual neural elements resulting from incomplete section?

This would seem an inappropriate assumption. All experimenters concerned were acutely aware of the necessity for complete transection in the mentioned spinal cord studies. The probability of all successful preparations to have escaped complete transection of

the spinal cord would be more than astronomical.

Thus from the foregoing historical review a number of features become apparent.

1) Central nervous system regeneration in mammalia is generally accepted.

2) It appears axiomatic that phylogenetic specificity is inversely proportional to the regenerative ability of central nervous neurons.

3) The glial scar is not the sole prohibitive factor to unimpeded central nervous regeneration. This scar can be modified by pyrogens, trypsin, Roentgen-ray radiation.

4) Intercostal nerve implants into the distal segment of the transected spinal cord potentiate functional recovery of paraplegic dogs.

It is with this background in mind that the present project was initiated to investigate the behavior of peripheral nerve implants in the distal segment of the transected spinal cord in dogs. More precisely, the potentials of these nerves in affecting functional recovery in experimental paraplegia in such animals was explored.

CHAPTER II

- METHODOLOGY -

Thirty-nine adult mongrel dogs of both sexes were used. Body weight ranged from 10 to 27 kg. Twenty-five of these dogs underwent a compulsory two week quarantine period prior to operation, during which interval they were immunized against canine distemper, dewormed and kept under close observation for signs of any disease.

The animals were separated into six experimental groups: -

I - Sham controls (2)

II - Controls (4)

III - One stage sympathetic trunk implant and spinal cord transection (11)

IV - As Group III, treated with ethanolamine - 1,2 (10)

V - Two-stage sympathetic chain implant and spinal cord transection (2)

VI - Single and multiple intercostal nerve implants (10)

A. OPERATIVE TECHNIQUES

Under intravenous pentobarbital sodium (Abbot) 30 mg./kg., the fasting animal was intubated, its back shaved and prepared with 80% alcohol-iodine solution. A continuous 5% Dextrose water intravenous drip was used throughout each operation.

GROUPS I and II (Sham controls and controls)

A longitudinal mid-dorsal incision was made extending approximately 5 vertebral segments from T-9 to L-2. Bleeders were secured and tied; subcutaneous tissues

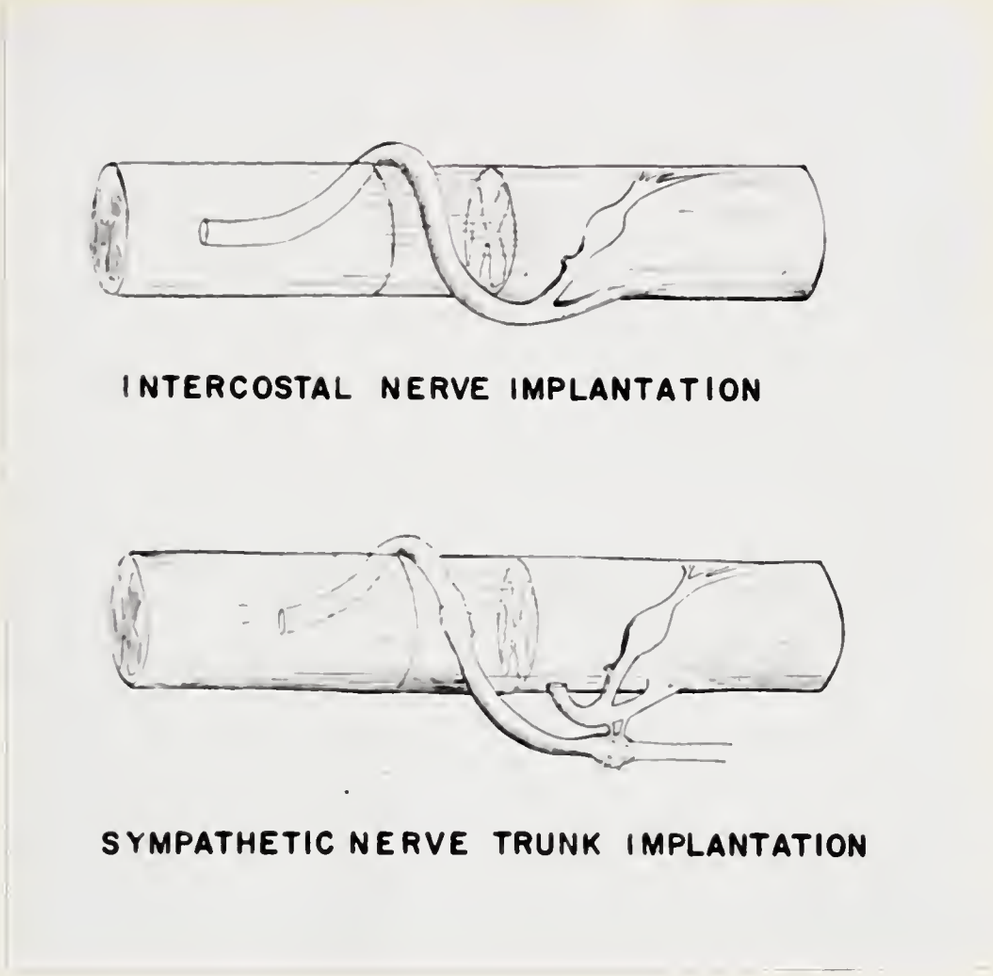

FIG. 1 - SCHEMATIC REPRESENTATION OF
NERVE IMPLANTATION PROCEDURES

incised over the vertebral spines and dissected with a scalpel handle. Three vertebral spines were removed with a Stille-Horsley bone cutter and laminectomy of two segments carried out by means of rongeurs. The level chosen was T-8 to T-10. The epidural fat was removed by gentle suction and the dura mater defined.

a) In sham controls, definitive surgery was terminated at this stage. Longissimus dorsi musculature was approximated using 0 chromic catgut, the subcutaneous tissue with 2-0 chromic catgut and skin edges held in apposition with alternating mattress and simple sutures, using 2-0 silk.

b) In controls, following removal of the epidural fat, the dura mater was incised longitudinally and its edges retracted by means of four 6-0 silk sutures. A 1 cm. segment of the spinal cord substance was excised de toto using iris scissors; hemorrhage checked by tamponade and the completeness of the excision ascertained. The edges of the dura mater were approximated, but no suturing was carried out to obtain this effect. Further closure was obtained in the manner already described.

GROUPS III and IV (One stage sympathetic chain implant; spinal cord transection)

A paravertebral incision was made 5 cm. from the midline, carried over ribs T-7 to T-13. Bleeders were secured, subcutaneous tissues dissected, and the natural cleavage plane between the lower border of the longissimus dorsi and the intercostalis externi extended

by blunt dissection. The longissimus dorsi was then retracted by means of two Weitlander retractors, exposing the lowermost four ribs and their intercostal muscle complex. Using a periosteal elevator, the periosteum of ribs T-10 - T-11 was resected in its entirety up to the costovertebral junction. A 3 - 5 cm. portion of each of these ribs was removed with the Stille-Horsley bone cutter, the intercostal neurovascular bundles defined, vessels ligated, the intercostalis externus and internus incised horizontally, and retracted. Using a Penfield No. 3 dissector, the pleura was gently dissected from its surrounding anatomy until the sympathetic trunk was defined. A 5 - 9 cm. portion of the sympathetic trunk was then dissected caudally and its rami communicantes (usually 2 - 3 pairs) cut, the main nerve trunk gently pulled away from its previous anatomical location and its end secured with a loose 6-0 silk sling knot.

If during the foregoing procedure a pneumothorax developed, the animal's respiration was assisted by a mechanical respirator and the pneumothorax reduced.

Following definition of the sympathetic trunk, laminectomy was carried out at T-8 - T-10 with excision of a 1 cm. portion of the spinal cord segment as previously described. The original skin incision was utilized for both sympathetic chain dissection and laminectomy. The lumbo-dorsal muscle mass was then tunnelled using a haemostat and the sympathetic trunk gently pulled into the

space created by the laminectomy. The end of the sympathetic nerve trunk was then introduced into the center of the substance of the distal segment of the transected spinal cord by means of a 6-0 silk suture. The nerve then was drawn through the spinal cord, its end emerging outside the dorsal aspect of the dura mater. The traumatized sympathetic nerve end then was severed and the nerve implant gently pulled back into the substance of the distal segment of the transected spinal cord.

The perineurium of the nerve implant was subsequently sutured to the edge of the incised dura mater with a single knot using 6-0 silk. Closure of the laminectomy exposure was obtained as described for Group I. This step was followed by closing the muscle separating exposure, using 2-0 chromic catgut. Skin was approximated as previously described.

GROUP V - (Two-stage sympathetic chain implant; spinal cord transection)

The operative procedure is technically identical to the one used in Group III, with the exception that the sympathetic trunk implant was inserted in the intact spinal cord at levels T-9 - T-10 in the first stage of the operation without previous spinal cord transection. The second stage of the operation was carried out two months later, when laminectomy at T-7 followed by transection of the spinal cord and removal of a 1 cm. segment of its substance was performed.

FIG. 2 - BILATERAL INTERCOSTAL NERVE IMPLANTS IN THE DISTAL SEGMENT OF THE TRANSECTED SPINAL CORD. NERVE ENDS ARE STILL SECURED WITH GUIDE SUTURES AND ARE DRAWN THROUGH THE DURA MATER.

GROUP VI (Single and multiple intercostal implants)

A transverse incision at T-11 was extended approximately 10 cm. to either side of the middorsal line. Laminectomy and transection of the spinal cord was identical to that in the sympathetic implant series. The intercostal nerve or nerves to be used as implants were defined one segment above the level of the lower end of the proximal stump of the transected spinal cord. The implant was mobilized by blunt dissection and divided about 5 cm. from the junction of the anterior and posterior spinal roots. The longissimus dorsi muscle was then tunnelled with a haemostat and the end of the proximal segment of the divided intercostal nerve drawn into the laminectomy exposure by means of a 6-0 silk suture. The implantation and closure was carried out as in Group III.

B. ETHANOLAMINE TREATMENT

Ethanolamine hydrochloride, C grade (calbiochem), M.W. 97.5, formula $HO(CH_2)_2NH_2.HCl$ was dissolved in sterile distilled water. Solution was prepared in such a concentration that one ml. of it represented a 100 mgm. aliquot part of ethanolamine. The animals received for 30 consecutive days subcutaneous injections of this solution, the dose being 20 mgm. ethanolamine/kg. body weight/day.

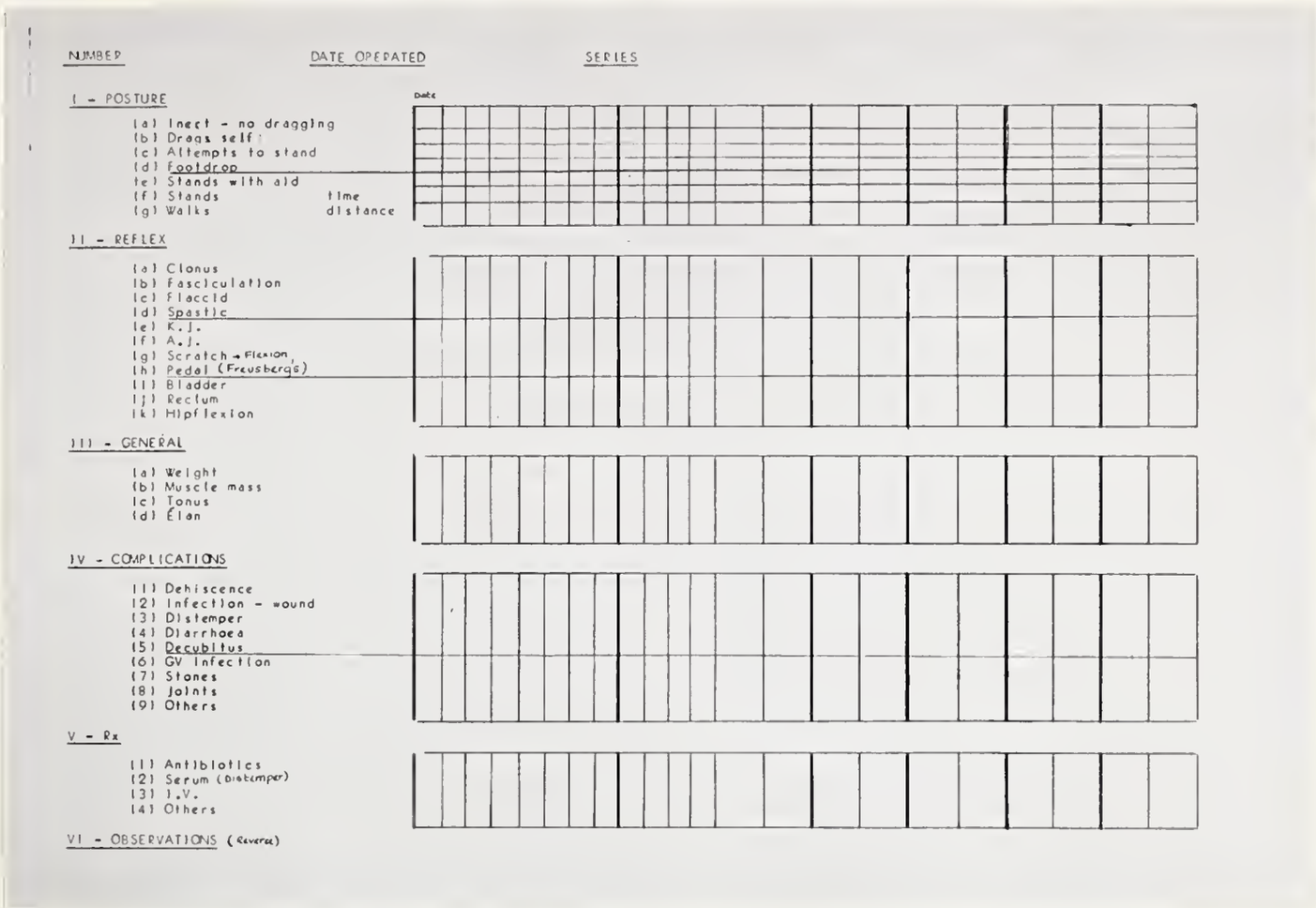

FIG. 3 - EVALUATION CHART

C. FUNCTIONAL EVALUATION

The animals were examined daily in the immediate postoperative period and then at one to two-weekly intervals. Normal and control dogs were assessed concurrently. Examination was carried out by one person but substantiation of equivocal findings was sought from other observers. No simple method for quantitative determination of functional recovery could be devised and evaluation was based on clinical observation and examination. Detailed charts were kept on each dog's performance.

D. ELECTROPHYSIOLOGICAL STUDIES

Under intravenous pentobarbital sodium anaesthesia an adequate laminectomy was carried out extending 2 - 3 segments from the site of nerve anastomosis. The spinal cord was clearly defined. The applicable nerve implant (sympathetic trunk or intercostal nerve or nerves) mobilized by blunt dissection using techniques similar to the initial operation performed. Care was taken not to traumatize the scar area. Silver wire stimulating electrodes were used. Recording electrodes were made of steel insect-mounting needles coated with Insul-x, placed 2 mm. apart and embedded in a plastic mount. The tips of the recording electrodes were cleared of insulation for the terminal 500 μ. with xylol.

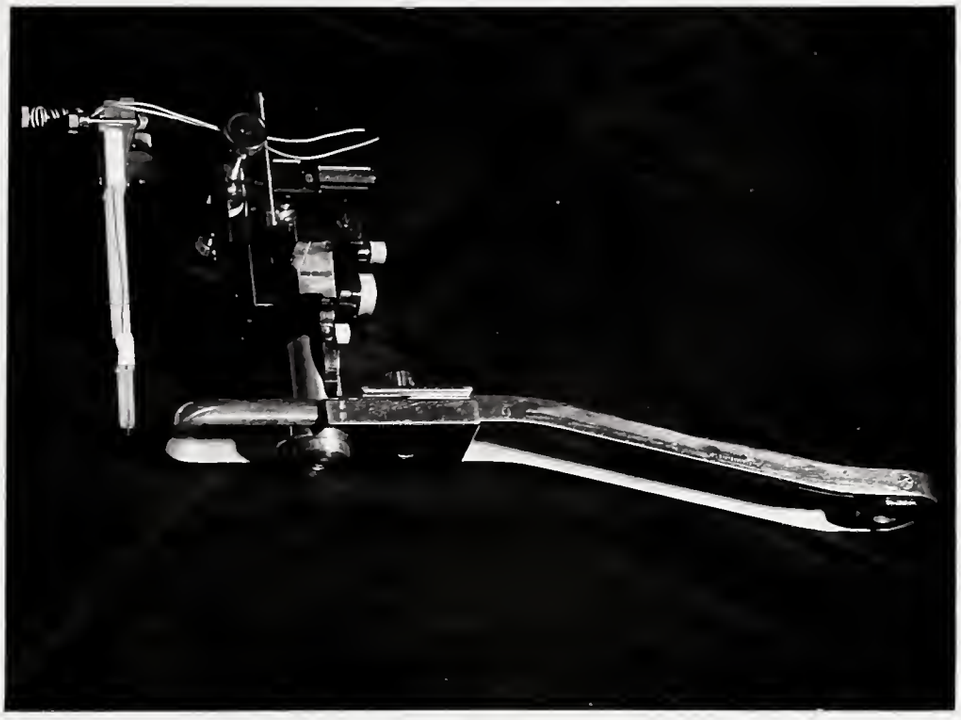

FIG. 4 - RECORDING ELECTRODES ATTACHED
TO STEREOTAXIC APPARATUS.

The stereotaxic mount designed could not always be positioned in such a manner that it would move synchronously with the animal's respiratory excursions, while holding the electrodes in a stationary recording locus. Thus a second small plastic mount was tried, attached directly to the animal's dorsal musculature.

A Grass stimulator S-4 connected to a Grass stimulus isolation unit SIU-4B and a P-5 Grass preamplifier was synchronized with a Nagard DT-103 oscilloscope; time base was fed in by a Heathkit audio-oscillator. Signals were also monitored by a loud speaker. Records were taken with a DuMont 2582-A camera. The stimulation experiments were conducted in a room protected by copper wire screen which precluded alternating current interference.

E. SECONDARY OPERATION - SECTION OF NERVE IMPLANT

Seven animals (6 sympathetic trunk implant and 1 intercostal nerve implant preparation) were reoperated 3 to 5 months after the initial nerve implantation. This operation was technically similar to the original procedures. The nerve implant was sectioned about 5 cm. proximal to the implantation site. In one operation (S-4) technical difficulties forced the termination of the procedure before the nerve implant could be sectioned. Dogs were observed daily for

functional activity; the level of function attained by the same animal prior to operation serving as a baseline.

F. HISTOLOGY

Animals were sacrificed using a lethal dose of pentobarbital sodium, and then perfused with formalin using the pulsatile perfusion technique of Haushalter and Bertram (1955). This method is an improved version of the method described by Koenig et al (1945). Following perfusion, a portion of the spinal cord was removed en bloc and placed in formalin. Seven to 10 days later the spinal cord was carefully dissected, the dura mater removed and the cord embedded in paraffin. Serial sections were stained using hematoxylin eosin, Bodian's protargol, Weigert's myelin stain techniques. The majority of the slides were stained using Peerson's modification of Bodian's protargol method for regenerating nerve fibrils. Aniline blue counterstain was occasionally employed. A number of sections of normal spinal cord and peripheral nerves were also prepared.

G. CARE OF ANIMALS

Animals were housed in the Vivarium of the University of Alberta. Three to four dogs were kept per kennel, which was lined with clean shavings to a depth of 6". Fecal material was removed daily and shavings changed every third day. Animals were fed commercial dog chow, with meat, milk and fat supplements;

water was always available. Dogs had daily manual expression of their bladders and the paralyzed limbs were put through a full range of motion by passive exercise. Complications such as infections, diarrhoea were promptly treated under the guidance of a qualified veterinarian. Five animals were cystoscoped and examined for evidence of neurogenic bladder reflux but no signs of this condition were discovered. One animal (C-1) had hysterectomy when the pregnant uterus produced urinary retention. The hydroureter was successfully relieved by the operation. Dog E-1 had exploratory laparotomy when it developed signs of progressive abdominal distention. No pathology was discovered and the animal had an uneventful recovery.

CHAPTER III

- O B S E R V A T I O N S -

A. CLINICAL OBSERVATIONS

The neurological status of normal animals served as a baseline for the functional evaluation of experimental animals.

The following grading of activity was employed:

a) Locomotor function

- Hind legs of the animal unable to support the weight of the body when the animal is placed in the standing position.

\+ Hind legs can support superincumbent body weight when animal placed in the standing position.

++ Animal able to stand unassisted.

+++ Animal able to stand unassisted; walks short distances. Front and hind limbs not co-ordinated.

++++ Co-ordinated walking ability.

N Normal locomotion.

b) Reflex function; spasticity

- Failure to elicit reflex; markedly diminished tonus.

\+ Normal reflex; normal tonus.

++ Increased reflex activity; medium spasticity.

+++ Hyperreflexia; hyperspasticity.

TABLE I

CONTROL ANIMALS

Number	Sex	Weight Kg	Date Operated	Date Sacrificed	Days Observed	Functional Grading	Earliest Appearance of Function in Weeks				Spasticity
							+	2+	3+	4+	
Control C1*	♀	12.4	28 IX 61	12 II 62	137	+	3				1+
C2#	♀	17.0	2 X 61		173 +	3+	4	5	24		3+
C3	♀	16.0	23 X 61	28 II 62	129	2+	5	7			1+
C4	♀	10.1	4 XII 61	8 II 62	66	-					1+
Sham Control SC1	♂	13.1	5 VII 61	7 IX 61	61	N	no neurological deficit				
SC2	♀	12.0	7 VII 61	7 IX 61	61	N	no neurological deficit				

* Hysterectomy
\# Kept for long term observation

TABLE I - CONTROL AND SHAM-CONTROL ANIMALS

1) Sham Controls

No neurological deficit was observed at any time during the two-month observation period.

2) Controls

Following operation a varying interval of depressed reflex activity was observed. This period lasted from one to six weeks, and was followed by a uniform hyperreflexic state. Foot drop, exaggerated flexion reflexes and brisk tendon reflexes were generally observed. While Freusberg's reflex was uniformly present at 5 weeks, crossed extension and extensor thrust phenomena were not always elicited. Pacing reflex in dog C-2, was observed between the 14th and 15th weeks. An interesting correlation was noticed between the simultaneous disappearance of the foot drop and the first appearance of the placing reflex, which involved the proper placing of the hind paws on the ground. These two features appeared from 4 to 9 weeks after operation. Stepping reflex was easily elicited at this time by lifting the caudal portion of the animal's body; hind legs performed rhythmic alternating flexion and extension movements. After a latency period of 6 weeks, three of the animals were able to support their weights, when they were placed in a standing position. At this time two dogs, C-2 and C-3, were also observed to stand up unassisted, taking a few steps. This act occurred in the following manner: From a sitting position the dog initiated movement by first flexing its head and

neck, at the same time internally rotating its forelimbs. The weight of the body was shifted forward and the hind legs were visibly raised off the ground without participating actively in this movement. The front limbs acted as a fulcrum for the torso. An apparent physical relationship was noted between the body configuration, front leg-body length ratio, and the facility with which the standing position was achieved. The abdominal musculature, serratus anterior and the powerful longissimus dorsi tensed up and virtually splinted the reflexly functioning distal segment of the body at an angle of 10 to 15 degrees above the normal horizontal body plane. The hind quarters barely touched the ground and when the paws came in contact with it, a reflex extensor response was thereby elicited. The hind legs then assumed more extensor tonus, and the anterior segment of the body released momentarily its almost spastic forward pull on the hind portion of the body. Pacing of one of the hind legs usually followed, but the corresponding front leg did not synchronize with this movement, remaining stationary in its initial position. The animal thus assumed a kyphotic habitus. The front limbs by necessity had to move forward to neutralize this further anterior displacement of the center of gravity of the body. If this accommodation was successful, forward locomotion or "spinal walking" resulted. In contradistinction, the animal lost balance and fell if the front limbs did not adjust to the new weight distribution by moving forward. Occasionally both hind legs

moved simultaneously, propelled by the extensor thrust reflex. In these instances, forward locomotion resembled a gallop. Dog C-3 frequently assumed a characteristic "tripod" stance. After standing up in the manner described, the hind paws came to rest close together while the front legs strained to keep the body upright. The animal was able to balance in this precarious position for a few seconds. This animal also exhibited hip flexion associated with the "tripod" stance.

Dog C-4 did not display any functional recovery. The thighs remained abducted and the animal maintained this "frog" position throughout the experiment. The animal dragged its passive hind legs, a trail of urine usually marking the path of locomotion.

A number of less frequently observed functional phenomena included spontaneous tail wag, flexion of the paws on pinching the plantar pad and flexion extension of the hind legs in response to tapping the tail with a reflex hammer. Stroking the tibial surface in dog C-1 also produced flexion of the ipsilateral paw. It was noted that during urination and defaecation the animals maintained their standing posture much more readily than during excretory quiescence. Some degree of bladder automaticity developed within one to two weeks postoperatively, while no apparent interference with faecal elimination was ascribable to the surgical procedure.

The muscle tonus in these animals was not excessive and never approached hyperspastic levels. Only a minimal amount of muscle wasting was noted in the hind limbs. Dorsal kyphosis as a result of operation was noticeable only in one dog (C-3).

3) Sympathetic chain implant preparations.

After operation, spinal shock, followed by depressed reflex activity persisted for 5 to 24 days. In four animals initial hyperreflexia, lasting 3 to 4 weeks, was followed by a diminution of reflex activity to levels slightly above normal. Subsequently two of these four dogs eventually exhibited co-ordinated front and hind leg movements, this locomotion essentially differing from that observed in controls. These animals achieved standing by simultaneous extension of the hind limbs and only a minimum amount of ancillary movement by the rostral portion of the body. Their forward locomotion resembled normal co-ordinated walking; sequential timing of the activity of front and hind limbs was very well synchronized. Dog S-4 was able to take up to 10 steps at a time before losing balance.

In five preparation differences in muscle tonus of the hind limbs were noted clinically. The side showing decreased tonus, as well as decreased quadriceps reflex excursion, corresponded to the side on which interruption of the sympathetic trunk was affected. In all of these five dogs, but most noticeably in S-7, this unequality of

TABLE II

SYMPATHETIC CHAIN IMPLANTATION

Number	Sex	Weight kg	Date Operated	Date Sacrificed	Days Observed	Functional Grading	Earliest Appearance of Function (wks)				Spasticity	Difference in Muscle Tonus	Side of Sympathectomy	Nerve Implant Section (day)	Electro-physiology	Histology	Notes
							+	2+	3+	4+							
S 1	♂	15.4	25 VII 61	19 II 62	209	+	6				3+	+	L			+	
S 2	♂	15.0	27 VII 61	10 II 62	211	4+	9	22	25	27	2+		L		+	+	
S 3	♂	11.6	26 VII 61	16 II 62	205	3+	4	6	6		3+		L		+	+	Functional deterioration 5 mons. post op.
S 4	♂	16.0	28 VII 61	15 III 62	221	4+	5	8	16	16	2+	+	L	208		+	
S 5	♂	27.0	31 VII 61	28 I 62	182	+	11				3+	+	L		+	+	
S 6	♂	17.2	1 VIII 61	15 II 62	199	3+	7	12	20		2+		L			+	
S 7	♂	16.4	2 VIII 61	15 III 62	226	3+	6	8	8		2+	+	L	203			
S 8	♀	10.5	4 VIII 61		239	3+ #	8	8	8		2+		L				Long term observation
S 9	♀	16.4	11 VIII 61	15 III 62	206	+	10				3+		L	185 +			Hind leg oedema
S 10	♂	15.3	16 VIII 61	15 III 62	201	3+ #	14	16	16		3+		L	171 +			
S 11	♀	12.7	23 VIII 61	20 I 62	150	- #					3+	+	L		+	+	"Frog" posture, clonus.
TS 1	♂	16.0	19 VII 61 19 IX 61	13 I 62	179	+	16				3+		R		+	+	Two stage operation
TS 2	♀	14.5	20 VII 61 19 IX 61	20 II 62	215	+	12				2+		R			+	Two stage operation

Hyperreflexia.

TABLE II - ANIMALS TREATED BY SYMPATHETIC NERVE TRUNK IMPLANTATION

tonus was so marked that the animal relied entirely on the contralateral hind limb for support of the caudal portion of the body. The crossed extensor reflex on the side where the sympathetic trunk remained intact was more pronounced, and was elicited by a smaller stimulus, than on the contralateral side.

Three preparations S-8, S-10, S-11, exhibited considerably more reflex activity than either the controls or the animals in the same experimental group. Flexion responses resulting from skin stimulation (scratch), as well as Freusberg's reflex, were hyperaccentuated. None of these animals showed clinical signs of true functional return.

Dog S-3, one of the earliest to show return of function, displayed definite signs of functional deterioation about 5 months postoperatively. While foot drop was absent at 4 weeks and the animal was able to stand and take a few steps 6 weeks after the operation, increased spasticity, return of foot drop and "braking" in the joints of the hind limbs was noted 20 weeks later. Adduction spasm, associated with persistent extension of the hind limbs was observed in one preparation (S-11); moderate hind leg oedema persisted in dog S-9. Priapism was frequently noted in this group of animals.

Bladder and bowel control did not differ from that observed in controls. The reflex and functional status, except for the mentioned phenomena, was essentially the same as in animals of the control group.

TABLE III

SYMPATHETIC CHAIN IMPLANTATION - ETHANOLAMINE TREATMENT*

Number	Sex	Weight kg	Date Operated	Date Sacrificed	Days Observed	Functional Grading	Earliest Appearance of Function (wks)				Spasticity	Difference in Muscle Tonus	Side of Sympathectomy	Nerve Implant Section (day)	Electro-physiology	Histology	Notes
							+	2+	3+	4+							
E 1	♀	13.7	20 IX 61	3 II 62	135	-					3+		L		+		"Frog" posture
E 2	♀	11.0	22 IX 61	7 III 62	157	+	6				2+	+	L	145			
E 3	♀	10.7	25 IX 61	18 III 62	175	-					2+		L			+	
E 4	♀	14.8	26 IX 61	26 II 62	154	2+	7	20	20		3+	+	L		+		
E 5	♂	10.1	3 X 61		175	3+	5	11	15		2+		L				Long term observation
E 6#	♀	10.2	3 X 61	15 III 62	161	4+	4	7	7	7	2+		L	144		+	
E 7	♀	10.3	16 X 61	28 II 62	135	+	7				2+		L			+	
E 8	♀	13.4	17 X 61	20 II 62	127	-					2+		L				
E 9	♀	10.8	19 X 61	17 III 62	150	3+	7	9	9		2+		L		+		
E 10	♀	10.5	20 X 61	5 III 62	135	3+	7	9	10		2+	+	L		+		

* Ethanolamine - 1, 2 hydrochloride (Calbiochem) 20 mg kg day for 30 consecutive days.
\# Complete functional regression noted following nerve section.

TABLE III - ANIMALS TREATED BY SYMPATHETIC NERVE TRUNK IMPLANTATION AND ETHANOLAMINE.

On one occasion, three animals (S-4, S-8, S-9) were given subcutaneous injections of adrenaline (1:1000) in increments of 1 ml. each dog receiving 3 ml. No changes in locomotor activity were observed following the injections.

4) Sympathetic chain implant preparations treated with ethanolamine

No noteworthy neurological differences were observed in this group from that of animals treated with sympathetic chain implantation only.

One dog, E-6, exhibited excellent functional recovery within two months after operation. This animal showed good co-ordination of front and hind limbs while walking distances up to 20 feet.

Differences in hind leg tonicity, already noted in the previous group, was observed in three dogs (E-2, E-4, E-10). The hind leg showing decreased tonus and decreased quadriceps reflex amplitude corresponded positionally to the side of sympathetic chain interruption.

Dog E-1 assumed the characteristic "frog" position described earlier. The hind legs were hyper-abducted and extended. Dislocation of the hip was queried, but x-rays did not show any defect in joint configuration. This dog subsequently developed arthrodesis of the involved joints, despite physiotherapy.

The subcutaneous injection of ethanolamine caused granuloma formation in four animals.

5) Two stage sympathetic chain implantation

In the postoperative period these animals exhibited no neurological deficit following insertion of the sympathetic chain. After the second stage of the procedure, both animals showed signs of complete spastic paraplegia. Both were able to support their weight in the standing position, but their functional recovery was arrested at this stage. Reflex activity showed no deviation from the pattern noted in controls. No variation in muscle tonicity was observed.

6) Intercostal nerve implantation

There were no noteworthy differences between the reflex activity of this group and that of controls. Spinal shock and depressed functional activity persisted from 3 to 8 weeks. Only dog I-3 demonstrated synchronized walking ability; this was observed three weeks after operation. This animal was injected at the site of nerve anastomosis with 2% procaine hydrochloride (Cutter) 145 days after the operation.⁂ Complete spastic paraplegia followed this maneuver. The animal was unable to support its weight in the standing position; reflexes were accentuated in the caudal portion of the body. Two hours after the injection, the dog again showed synchronized walking ability for distances up to 10 feet.

Three dogs (E-2, E-4, E-10) assumed the described "frog" position. The hind legs of these preparations were hyperextended and remained "frozen" in that position.

⁂ No C.S.F. was aspirated prior to injection

TABLE IV

INTERCOSTAL NERVE IMPLANTATION

Number	Sex	Weight kg	Date Operated	Date Sacrificed	Days Observed	Functional Grading	Earliest Appearance of Function (wks)				Spasticity	Number and Side of Implant	Nerve Implant Section (day)	Electro-physiology	Histology	Notes
							+	2+	3+	4+						
I 1	♀	11.4	30 X 61	12 III 62	134	-					3+	R.L		+		
I 2	♀	9.0	6 XI 61	20 II 62	106	-					3+	R.L			+	"Frog" posture
I 3=	♂	8.5	7 XI 61		150	4+	3	4	4	4	2+	2R.L				Long term observation
I 5	♀	12.8	10 XI 61	7 III 62	117	2+	7	7			3+	R.L	107			
I 6	♀	11.5	13 XI 61	20 II 62	100	-					3+	R.L			+	"Frog" posture
I 7	♀	10.8	14 XI 61	3 III 62	110	+	8				2+	R.L		+		
I 8*	♀	13.0	16 XI 61		136	3+	8	11	13		2+	R.L				Long term observation
I 9	♀	11.4	17 XI 61	7 III 62	111	+	6				3+	R.L	103			
I 10	♀	13.5	20 XI 61	7 III 62	108	-					2+	2R.L			+	
I 12	♀	13.0	28 XI 61	28 II 62	93	-					3+	L				

* Had choreiform movements.
\# Procaine block produced reversible paraplegia 145 days after operation.

TABLE IV - ANIMALS TREATED BY INTERCOSTAL NERVE IMPLANTATION

FIG. 5

Dog I-3, before and after procaine block of intercostal nerve implant.

(BEFORE)

(AFTER)

Dog I-8 developed choreiform movements of the whole body after operation. This condition lasted for two weeks and could be controlled only with tranquilizers. Daily intravenous therapy had to be instituted; antibiotic therapy had no effect in reducing the chorea. Subsequently good functional recovery was observed in this dog.

7) Nerve implant section

Eight representative animals were operated; five operations were considered as technically successful ones. Two animals, S-7 and S-10 could still stand after the operation but were unable to demonstrate stepping and pacing phenomena. Two animals, S-9 and E-2, showed no change in functional status. Animal I-9 could not support its weight after the operation.

Dog E-6, which showed co-ordinated front and hind leg movements prior to operation reverted to complete paraplegia after nerve implant section. It was unable to stand or support the weight of the caudal segment of the body on its hind limbs. The surgical procedure of nerve section lasted only $1\frac{1}{2}$ hours and the trauma was minimal. The dog was observed for three weeks following nerve implant section but no change in functional activity was noted.

B. ELECTROPHYSIOLOGICAL STUDIES

Ten animals were examined electrophysiologically to see if stimulation of the nerve implant, 5 cm. above the implantation site, could elicit evoked potentials in the

distal segment of the transected spinal cord. The technical apparatus was frequently tested and the stimulating and recording electrodes were found to be adequate. Stimulation studies were carried out to determine the nature of induced compound action potentials. Muscle activity associated with respiration was controlled in one experiment by injecting succinylcholine intravenously.

DOG E-4 - Recording electrodes appr. 3 mm. below the implantation site. Sympathetic trunk stimulated 4 - 5 cm. above the implantation site.

a) Spontaneous nervous activity was recorded.

b) Evoked potentials recorded on stimulation.

c) Crushing of the sympathetic trunk distal to the stimulating electrodes was followed by temporary absence of evoked potentials. Some induced nervous activity was subsequently recorded with electrodes remaining in the same position.

DOG I-7 - Recording electrodes 2 mm. below nerve implantation site. Intercostal nerve stimulated 4 - 5 cm. above the implantation site.

a) Spontaneous activity recorded in the distal segment of the spinal cord.

b) Compound action potentials recorded following stimulation. These action potentials decreased in magnitude and then were not observed when

TABLE V

STIMULATION STUDIES

Dog Number	Days after Operation	Spontaneous Action Potentials in Distal Segment of Spinal Cord	Evoked Potentials	Nerve Implant Severed	Evoked Potentials After Nerve Implant Severed	Photographic Records	Notes
TS 1	179	-	-	No	-	-	Negative experiment
S 2	211	-	-	No	-	+	Negative experiment
S 3	205	+	±	Yes	±	+	Cf. Figure 6
S 5	182	+	±	No	-	+	
S 11	150	-	-	No	-	+	Negative experiment
E 4	154	+	+	Yes	±	+	Cf. Figure 7
E 9	150	±	-	No	-	-	Negative experiment
E 10	135	+	+	No	-	+	Assisted respiration Succinylcholine block Cf. Figure 8
I 1	134	-	-	No	-	-	Negative experiment
I 7	110	+	+	Yes	-	-	Technical failure of Recording Camera

TABLE V - RESULTS OF STIMULATION STUDIES

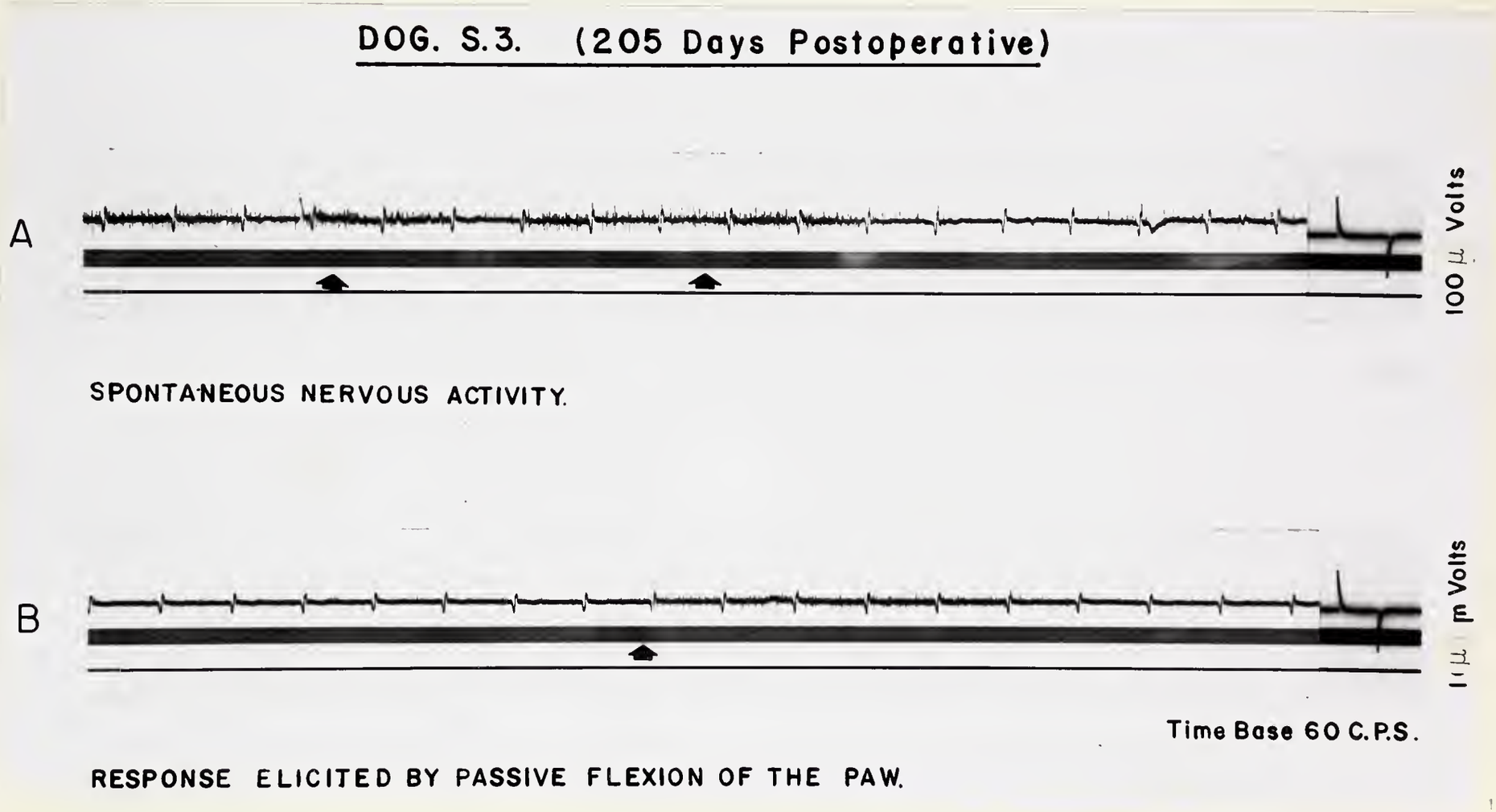

FIG. 6 - SPINAL CORD - OSCILLOGRAPHIC RECORDING 3 MM. BELOW SITE OF NERVE IMPLANTATION

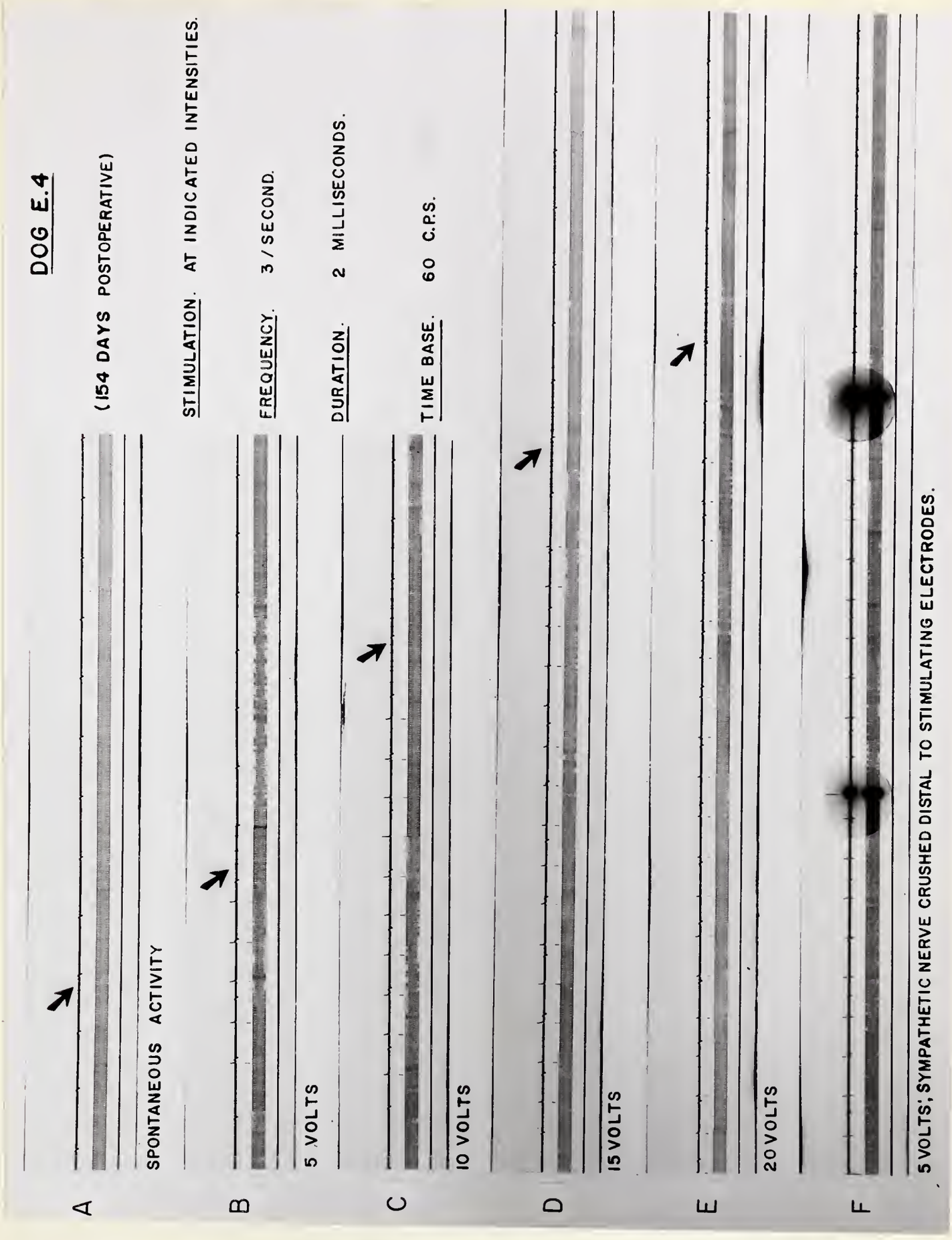

FIG. 7 - SPINAL CORD - OSCILLOGRAPHIC RECORDING 3 MM. BELOW SITE OF NERVE IMPLANTATION.

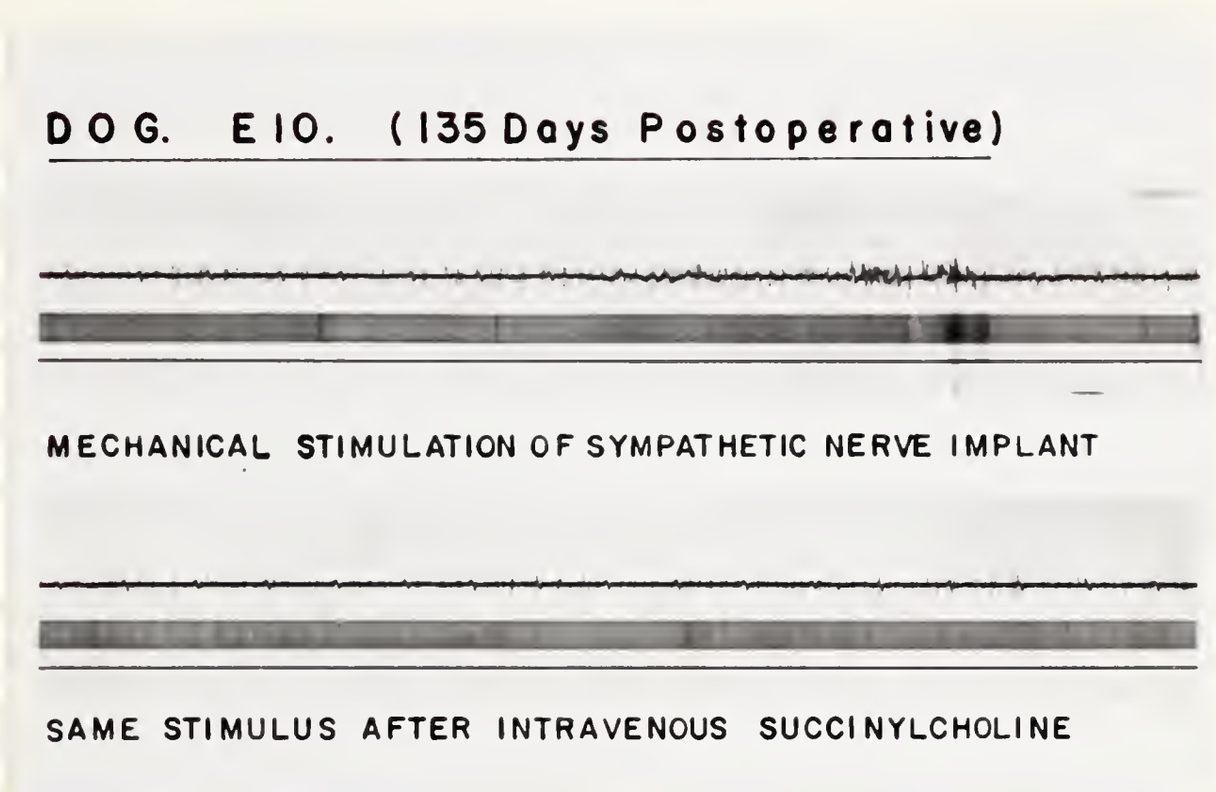

FIG. 8 - DOG E-10. OSCILLOGRAPHIC RECORDING FROM IMPLANTATION SITE.

the recording electrodes were inserted 5 mm. and 2 cm. respectively, caudal to the implantation site.

c) Section of the nerve implant distal to the stimulating electrodes resulted in absence of evoked action potentials 2 mm. below the nerve implantation area. The records of this experiment were not salvaged due to technical failure of the recording camera.

DOG E-10 - Mechanical tension on the sympathetic trunk 5 cm. above the implantation site produced evoked action potentials in the distal segment of the spinal cord. Electrical stimulation produced comparable results. When the animal was given intravenous succinylcholine, only a few action potentials were recorded with the same stimulus parameters.

C. MICROSCOPIC ANATOMY

The available histological sections confirm:

(1) Complete transection of the spinal cord.

(2) Regeneration of the peripheral nerve implant in the distal segment of the spinal cord.

Figure 11 shows the terminal portion of a sympathetic nerve implant in dog S-1. Terminal bouton formation and a cone composed of actively regenerating

FIG. 9 - MICROSCOPIC SECTION SHOWING COMPLETENESS OF SPINAL CORD TRANSECTION IN CONTROL DOG C-3. (MAGNIFICATION 3 x.) WEIGERT'S MYELIN STAIN.

neurofibrils is evident.

The histological studies are not yet complete but the critical features, viz. complete spinal cord transection and nerve implant regeneration are clearly demonstrated.

D. MORTALITY

Of the 59 animals operated, 11 died or were sacrificed before standardization of techniques was achieved. Nine animals died following this period. The compulsory two-week quarantine period prior to operation was introduced in August.

Animals which were sacrificed or died during this project were excluded from the study. Mortality figures are tabulated in Table VI.

FIG. 10 - ACTIVELY GROWING NEUROFIBRILS OF SYMPATHETIC NERVE IMPLANT IN THE DISTAL SEGMENT OF THE SPINAL CORD (DOG S-1).

(BODIAN'S PROTARGOL, MAGNIFICATION 500 x)

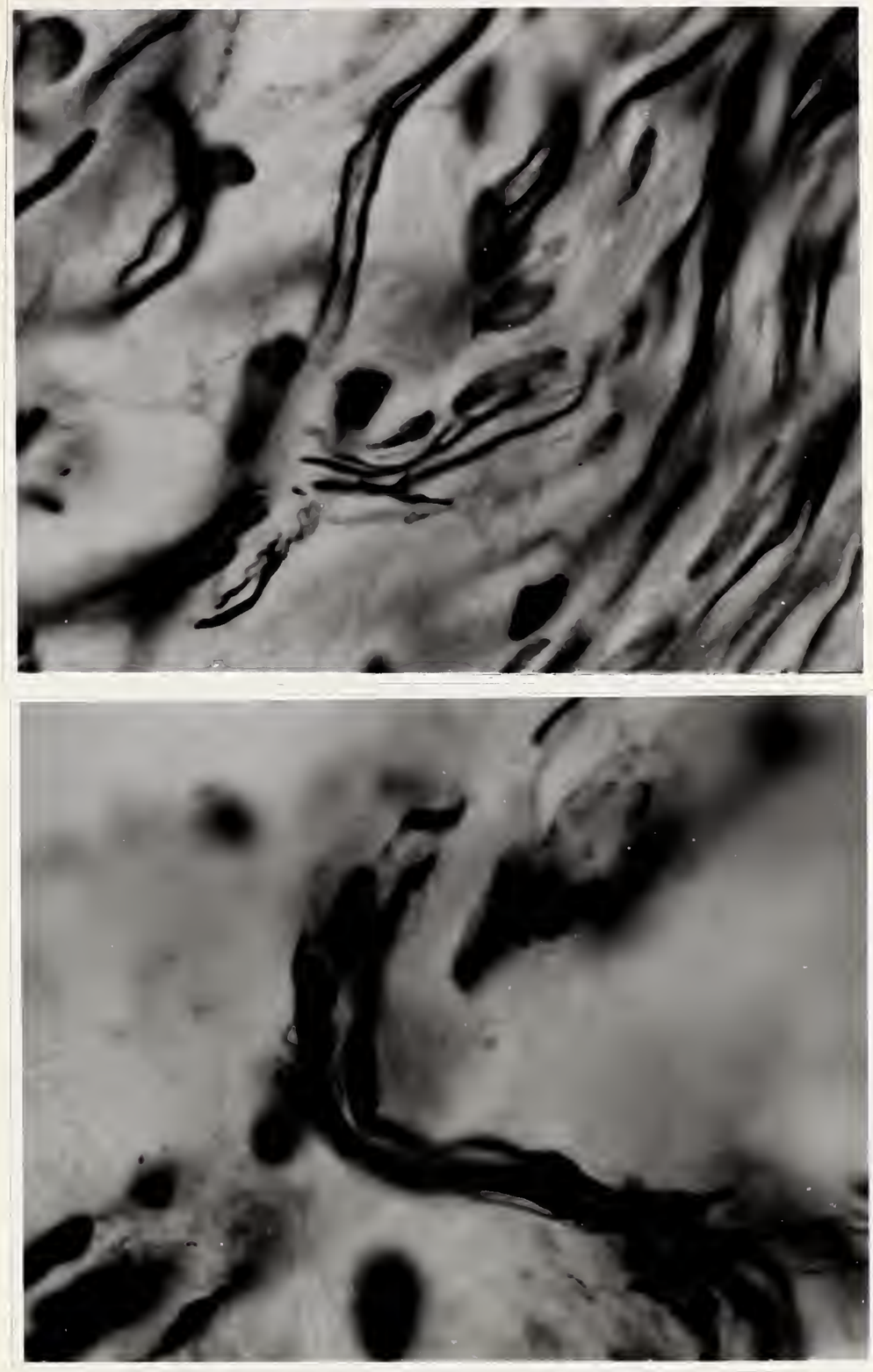

FIG. 11 - OIL IMMERSION PHOTOMICROGRAPH
OF SECTION SHOWN IN FIG. 10.
(MAGNIFICATION 1250 x)

TABLE VI

MORTALITY FIGURES

Dog Number	Date Operated	Died or Sacrificed	Number of Days After Operation	Pathology	Notes
1	28 VI 61	Sacrificed	5	Decubitus ulcer	Inadequate quarters
2	29 VI 61	"	14	Wound infection	
3	30 VI 61	"	5	Wound infection	
6	10 VII 61	Died	At operation	-	Shock?
7	18 VII 61	"	1	-	Shock?
8	19 VII 61	"	2	-	Shock?
9	20 VII 61	"	2	Ileus, Peritonitis	
10	21 VII 61	"	24	Distemper	
11	25 VII 61	"	25	Distemper	
12	26 VII 61	"	10	Distemper	
13	27 VII 61	"	28	Pneumonia	Secondary to wound infection
18	3 VIII 61	Sacrificed	At operation	-	Technical failure
20	9 VIII 61	Died	2	-	Shock?
21	10 VIII 61	"	4	Distemper	
23	14 VIII 61	"	17	Distemper	
26	24 VIII 61	"	24	Distemper	
34	4 IX 61	"	At operation	-	Technical failure
42	3 XI 61	"	10	Pneumonia	
45	8 XI 61	"	12	Pancreatitis	
52	23 XI 61	"	3	-	Shock?

TABLE VI - MORTALITY DATA

CHAPTER IV

- D I S C U S S I O N -

This experiment was based on the assumption that centrally connected peripheral nerve implants in the distal segment of the transected spinal cord are capable of influencing the functional activity of the paraplegic animal. The hypothesis also presupposed that this influence is exerted by regenerating fibers of the nerve implant.

One now has to resolve:

1) Whether the functional return noted in some of the experimental animals was purely on a reflex basis.
2) Whether observed differences in functional activity between control and treated animals is ascribable to nerve implantation.
3) Whether regeneration of the nerve implant occurred and the manner in which it influenced the reflex activity of the spinal cord.

The various treatment parameters also must be examined.

Sherrington (1910) analyzed the limb reflexes of decerebrate, decapitate and spinal preparations in a most comprehensive communication. His conclusions, as well as observations by Ranson and Hinsey (1930) and McCouch (1947) support the findings of this study concerning spinal preparations. Thus, standing, stepping and a type of forward locomotion resembling walking may be observed in spinal dogs; however, co-ordinated walking is not demonstrated by such animals. Sherrington in the same study comments on the locomotion of decerebrate dogs, "Reflex

stepping movements of the limbs even when including all four limbs timed in appropriate sequence yet of themselves alone do not constitute a complete reflex act of walking or running. For this they must be duly combined with the general static reflex maintaining erect posture of head and neck."

On this basis, in this study only preparations which conformed as closely as possible to this concept of normal walking were considered as having demonstrated functional recovery. Four dogs showed locomotor activity sufficiently different from that of control animals to warrant the conjecture that nerve implantation alone could be responsible for this difference. One may justifiably ask then why not all of the similarly-treated animals demonstrated such return of function. The various parameters to be considered are numerous and include among others biological variation in tissue response, viability of nerve implant, milieu of the implantation site, type and number of nerve implants used, surgical technique, condition and response (élan) of the animal.

Before accepting the thesis that nerve implantation did in fact account for the improved functional performance of the animals, further corroborating evidence must be advanced. As will be recalled, procaine block of the nerve implantation site in animal I-3 did produce

reversible regression in function. Not only was this dog unable to walk in a co-ordinated fashion, but it could not even support its body weight in the standing position. Also, section of the nerve implant in dog E-6 produced complete paraplegia, while in dog S-7 increased spasticity and functional regression was noted. One may query whether surgical trauma associated with section of the nerve implant could have adversely influenced the animal's functional performance, thus accounting for the altered behavior. This operation however was only of short duration, the trauma minimal and the recovery period adequate.

In a number of animals the stimulation studies clearly demonstrated spontaneous activity plus evoked potentials in the implantation area. In dog I-7, section of the nerve implant was followed by absence of induced potentials in the distal segment of the spinal cord. The induced activity in animal S-3 showed an interesting pattern. Stimulus was not followed immediately by an evoked response, a short latency period was observed between the cessation of stimulation and the volley of nervous discharge recorded. The number of stimuli showed an almost linear relationship to the duration of induced activity. It is possible that a recruitment phenomenon accounted for this response.

The possibility of stimulus leak producing

simultaneous muscle stretch receptor activity in the distal spinal cord has to be considered. While precautions were taken to minimize such a phenomenon from occurring, there is a remote possibility that stretch receptor activity may have been recorded. In view of this fact one can advance the electrophysiological findings only as circumstantial proof for reinnervation of the distal stump of the spinal cord.

Cortical stimulation with myographic recordings in the caudal portion of the body is considered in future preparations.

The advanced body of evidence per se, strongly suggests that the nerve implantation procedure does in fact have a demonstrable beneficial effect on the locomotor function of spinal animals. One must postulate that regenerating neurofibrils of the nerve implant reinnervate the distal spinal cord segment, thereby effecting improved function.

The existence of viable neurofibrils growing from the nerve implant was demonstrated. It is interesting, that no reinnervation was observed in histological sections of animals which showed no signs of functional recovery. That peripheral nerve implant regeneration proceeds unimpeded by scar tissue indicates that Piromen and Roentgen-ray therapy (Jakoby et al 1958) have only a quantitative, rather than qualitative effect on such regeneration.

How could regenerating nerve fibrils of the nerve implant influence the functional behavior of spinal animals? In the case of intercostal nerve implants a plausible theory would be to assume that neural continuity is established between higher centers and the reflexly functioning distal spinal stump by regenerating fibrils creating functional synapses with motor elements. Freeman (1961) concerning intercostal nerve implants states, "It is apparent that the growing tip of axons exude a material which either excites or inhibits the chemical mechanisms which are responsible for the reflex activity of the motor and sensory pools in the distal-divided stump of the spinal cord." Enzyme histology indicated "vast quantities of chemicals commonly associated with neuronal activity." It is likely that such a biochemical mechanism operates following sympathetic chain implantation. Preganglionic sympathetic fibers could be activated in the proximal segment and its regenerating terminal end in the distal segment of the cord likely could affect the biochemical milieu. The neuropile thus may be influenced in such a way that the unequal balance between flexor-extensor activity is restituted to normal equilibrium. Hyperspasticity was never observed in any of the animals which showed functional return. While the proposed mode of action of the regenerating neurofibrils is inferential, its existence must be accepted and the exact mechanism as well as the site of this phenomenon should be further

investigated.

No significant differences were noted in the functional behavior of animals treated by sympathetic chain implantation and intercostal nerve insertion. On this basis, a similarity in the mechanism of their action must be assumed. Multiple intercostal nerve implantation did not adversely affect the functional recovery of the animals thus treated. No functional or histological differences were attributable to ethanolamine therapy.

Validation of observed differences in muscle tonicity following sympathetic deafferentation is found in the work of Kuntz and Kerper (1926), Phillips (1931) and Spychala (1932).

The observation, that a myotatic contraction follows the quadriceps reflex in the sympathetic denervated limb, was made by Orbeli, "das sympathetisch entnervte Bein dazu neigt, tonisch in Streckstellung zu verbleiben und dasz es rascher ermüdet" (cf. Spychala 1932). While differences in muscle tonus in dogs with sympathetic deafferentation but intact spinal cords was apparent only during anaesthesia (Kuntz and Kerper 1926), this feature was observed in the present experiment clinically in 9 of the dogs which had spinal transection in addition to sympathetic trunk section.

CHAPTER V

- C O N C L U S I O N S -

Centrally connected intercostal nerves and sympathetic nerve trunks were used as implants in the distal segment of the transected spinal cord in 33 dogs. Six dogs acted as controls.

On the basis of functional, electrophysiological and histological studies the following conclusions are warranted:

1) After complete transection of the spinal cord at T-10, dogs may show various locomotor phenomena, but co-ordination of front and hind limbs is absent.

2) The intercostal and sympathetic chain implants appear to be responsible for the improved functional performance in the surgically treated paraplegic animals.

3) Ethanolamine therapy produced no demonstrable changes under the described experimental conditions.

4) The nerve implants can regenerate and are capable of nerve impulse conduction.

5) Sympathetic deafferentation of the hind limb of a paraplegic dog with high spinal cord transection is followed by decreased muscle tonicity and changes in the reflex activity of that limb.

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A P P E N D I X

The staining techniques used were designed to demonstrate neurofibrillae preferentially to other neural elements. Bodian's protargol method (Anat. Rec., 69;153-162, 1937) is especially suited for this purpose.

Regeneration of the nerve implant was admitted to be present when its terminal growth cone demonstrated the features of collateral and terminal neoformation described by Cajal (1928).

The plus sign in Tables II, III and IV, appearing under the heading "Histology" indicates that the spinal cords of these animals were examined in serial histologic sections; this included to date, two control dogs and ten animals treated by peripheral nerve implantation.

Technically perfect histological preparations were obtained in only four of the group of ten dogs indicated. In these sections, the peripheral nerve implant was demonstrated in the distal segment of the spinal cord in dogs S-1 and S-11. Regeneration of the nerve implant could be ascertained on a histologic basis in dog S-1 only.